Kidney International, Vol. 40 (1991), pp. 514–524

Histopathologic findings associated with a chronic, progressive decline in renal allograft function

BERTRAM L. KASISKE, ROBERTO S.N. KALIL, HYUN SOON LEE, and K. VENKATESWARA RAO

Department of Medicine, University of Minnesota College of Medicine, Hennepin County Medical Center, Minneapolis, Minnesota, USA; Department of Medicine, Evangelic School of Medicine, Curitiba, Paranà, Brazil; and Department of Pathology, Seoul National University, Seoul, South Korea

Histopathologic findings associated with a chronic, progressive decline in renal allograft function. The relationship between specific histopathologic findings of chronic rejection (CR) and the clinical course of renal transplant recipients with a chronic progressive decline in allograft function (CPDAF) is unknown. We used one or two hinged regression lines, fitted by least-squares to serial creatinine clearances, to define the onset and clinical course of CPDAF. Biopsies ($N = 100$) from patients transplanted from 1978 to 1982 were studied retrospectively. Interstitial fibrosis, tubular atrophy, and fibrointimal arterial narrowing were more pronounced in biopsies obtained after, but not before the onset of CPDAF. Interstitial hemorrhage, an infrequent finding in acute vascular rejection, preceded the onset of CPDAF, but the more common histologic findings of acute cellular rejection did not. The severity of histologic features of CR (as reflected by a score combining fibrointimal arterial narrowing, interstitial fibrosis, tubular atrophy, glomerular sclerosis, glomerular mesangial expansion, and glomerular basement membrane reduplication) correlated with the duration of subsequent allograft survival ($r = -0.65$, $P < 0.001$). Glomerular size increased after transplantation, but was not different in patients with or without CPDAF, suggesting that mechanisms related to compensatory hypertrophy did not play a major role in the pathogenesis of CR. In summary, the histologic findings of CR did not predict the onset of CPDAF, did not distinguish whether the pathogenesis was mediated by immune or nonimmune events, but did correlate with the duration of subsequent allograft survival.

Acute renal allograft rejection is characterized by a rapid deterioration in function and histologic findings that often predict the clinical course and the response to treatment [1–6]. Chronic rejection (CR) is histologically distinct from acute rejection [7–12], and responds poorly to treatment [12–15]. Although combined patient and allograft survival was recently shown to be reduced in patients with CR [5], few studies have systematically examined the clinical course of patients whose allograft biopsy showed CR. As a result, several questions remain unanswered. Specifically, it is not known how often the histologic features of CR precede, and thus potentially predict a chronic, progressive decline in renal allograft function (CPDAF). It is possible that the histologic findings of CR may only occur after a substantial amount of function has been lost, in which case such findings would have little predictive value. Also unclear is how well the histologic findings of CR correlate with the subsequent functional decline, and whether CR is compatible with long-term graft survival. In this regard, some descriptions of CR were from patients who lost their grafts within the first few weeks after transplantation [7], suggesting that a biopsy diagnosis of CR may not always imply that the subsequent clinical course will be chronic. Finally, the frequency with which hypertension, proteinuria, and other clinical parameters precede or accompany the histologic findings of CR has not been well documented.

We used serial creatinine clearances (C_{Cr}) and curve-fitting techniques to define the incidence, time of onset, and clinical course of CPDAF in patients who survived with a functioning allograft for at least one year after transplantation [16]. In the present study, the results of biopsies obtained before and after onset of CPDAF were compared with those from biopsies obtained early and late in the course of control patients who had only acute declines, or maintained stable allograft function. In addition, the relationship between renal function, hypertension, proteinuria and specific histologic findings of CR were examined.

Methods

Patients

The 100 biopsies analyzed in the present study were obtained from 74 patients included in a larger group of transplant recipients whose clinical course has previously been described [16]. All patients were transplanted between January, 1978, and August, 1982, and had allografts that functioned for one year or longer. Patients were followed until January 1, 1990. The immunosuppression protocol utilized Minnesota antilymphocyte globulin, corticosteroids, and azathioprine, as previously described [16]. All but one patient had undergone splenectomy prior to transplantation, and all but two transplants were from cadaver donors. None of the patients were treated with cyclosporine.

Allograft function

The baseline C_{Cr} was measured 5 ± 4 months before biopsy. The change in C_{Cr} immediately before biopsy was calculated as the difference between the C_{Cr} at the time of biopsy and the baseline C_{Cr}. The change in C_{Cr} by 5 ± 4 months after biopsy

Received for publication November 13, 1990
and in revised form April 18, 1991
Accepted for publication April 19, 1991

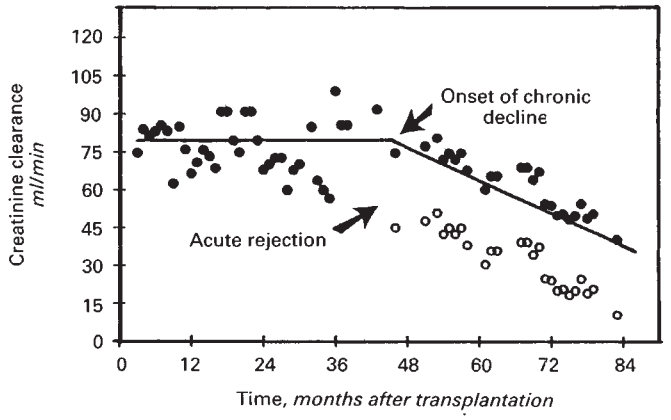

Fig. 1. *The clinical course of a typical patient with a chronic, progressive decline in allograft function is shown.* The patient had an acute, irreversible decline in C_{Cr} 46 months after transplantation. The function lost during this acute rejection episode was added back to the baseline C_{Cr} to permit an analysis of chronic changes in renal function. The open circles show the actual C_{Cr} values after the acute decline in function. The solid circles show the C_{Cr} values before the acute rejection episode, and the C_{Cr} values corrected for the acute, irreversible decline in function that was associated with the acute rejection episode. Regression analysis showed that two hinged lines fitted the data better than a single line, and that the onset, or breakpoint, occurred at about the time of the acute rejection episode.

was calculated as the difference between the C_{Cr} after biopsy and the C_{Cr} at the time of biopsy. Chronic trends in allograft function were analyzed using C_{Cr} and interim serum creatinine levels as previously described [16]. The serum creatinines for this analysis were obtained monthly. The C_{Cr} measurements were made routinely at 3, 6, 9, 12, 18, 24 months, and yearly thereafter. Additional C_{Cr} measurements were obtained when there was a change in serum creatinine. After correcting for acute, irreversible changes in C_{Cr} that occurred within a three month time period, the chronic course of declining renal function was modeled as the best 1, or 2, least-squares-fitted, hinged, regression lines using the method of Jones and Molitoris (Fig. 1) [16, 17].

Biopsies

At our center, the first acute decline in allograft function (manifested by at least a 30% decrease in C_{Cr}) was empirically treated with intravenous corticosteroids. A decline in function that did not reverse with treatment, or a second decline in function (acute or chronic) was used as an indication for biopsy. In addition, biopsies were also obtained for urine protein excretion greater than 1.0 g/24 hr. Cases of recurrent or secondary glomerular disease, for example, type II membranoproliferative glomerulonephritis, diabetic nephropathy or systemic amyloidosis, were not included in the analysis. Of the 100 biopsies that were studied, 44 were from 29 patients with CPDAF and 56 were from 45 control patients who had a stable clinical course or an acute decline in function. For analysis, the biopsies were divided into four groups: 1) 13 biopsies were obtained before the onset of CPDAF (within the first nine months after transplantation); 2) 31 were obtained after the onset of CPDAF; 3) 11 biopsies were obtained within the first nine months after transplantation in control patients who did not have CPDAF; and 4) 45 were obtained later in the post-transplant period in patients who did not have CPDAF.

Renal histology

Tissue for light microscopy was fixed in 10% buffered formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin, Masson's trichrome, and Jones' modification of the periodic acid-methenamine silver stain. All biopsies were examined independently, and in a blinded fashion, by two investigators. For each specimen, 16 histologic features were evaluated. For each feature, the investigator indicated the degree of involvement by placing a mark on a continuous 0 to 100 scale. On this scale, 0 represented normal, or no involvement, and 100 represented the greatest amount of involvement. For each individual histologic parameter, a mean score was computed from the separate scores of the two investigators.

The individual features evaluated were: 1) interstitial edema; 2) interstitial fibrosis; 3) acute, interstitial, mononuclear cell infiltration; 4) interstitial hemorrhage; 5) glomerular capillary endothelial swelling and proliferation; 6) glomerular mesangial expansion; 7) glomerular polymorphonuclear cell infiltration; 8) necrosis of glomerular tufts; 9) vascular (arterial or arteriolar) necrosis; 10) vascular endothelial cell swelling and proliferation; 11) chronic, fibrointimal arterial lumen encroachment; 12) fibrin thrombi in glomerular or interstitial capillaries; 13) chronic tubular atrophy and loss; 14) glomerular basement membrane splitting, or reduplication; and 15) epimembranous deposits. In addition, the percent of glomeruli which were completely hyalinized was determined.

Mean glomerular areas were determined using planar morphometry. The perimeter of Bowman's capsule was traced using a video camera and a computerized, digital image analysis system (Southern Micro Instruments, Inc., Atlanta, Georgia, USA). The integrated area within the perimeter was then computed. The mean glomerular area was the arithmetic mean of the individual measurements in each specimen. Sclerotic glomeruli were not measured.

Immunofluorescence, and electron microscopic studies were also available in most cases. The results of these studies were used to confirm specific biopsy findings noted on light microscopy, such as, the presence or absence of epimembranous deposits. Thus, immunofluorescence and electron microscopy results were used only indirectly in the semiquantitative scoring and analysis.

Hypertension and proteinuria

Blood pressure was measured 5 ± 4 months before, at the time of, and 5 ± 4 months after biopsy. Blood pressure was expressed as mean arterial pressure: (MAP) = (⅓ × (systolic-diastolic)) + diastolic. For patients receiving antihypertensive medications, blood pressure measurements alone could not adequately reflect the degree of hypertension. Therefore, a Hypertension Index was calculated to take into account both the MAP and the number of antihypertensive medications that were being used when the MAP was measured: Hypertension Index = N + [(MAP − 93)/20], where N was the number of different types of antihypertensive medications, including diuretics, being used. Thus, for a patient receiving no antihypertensive medications whose blood pressure was 120/80 mm Hg (MAP = 93 mm Hg) the Hypertension Index was 0. At the same

time that C_{Cr} and blood pressure were determined, 24-hour urine protein excretion was also measured.

Statistical analysis

Each study variable was examined using indices of kurtosis and skewness, and the Kolmogorov-Smirnov test to determine which distributions were Gaussian. Variables that required logarithmic transformation to achieve a normal distribution prior to analysis included the glomerular area and glomerular polymorphonuclear cell infiltration. Variables that were not normally distributed, even after appropriate transformation, included the percent of glomeruli with partial or total sclerosis, interstitial hemorrhage, capillary endothelial cell swelling and proliferation, necrosis of glomerular tufts, vascular necrosis, vascular endothelial cell swelling and proliferation, capillary fibrin thrombi, glomerular basement membrane splitting, and epimembranous deposits. However, when these non-Gaussian measures were added to other Gaussian and non-Gaussian measures, the resulting composite scores were normally distributed.

The significance of differences between groups was assessed using Student's *t*-test or, in the case of multiple groups, one way analysis of variance (ANOVA) with Duncan's multiple range comparison test. To test group differences for non-parametric variables, the Kruskal-Wallis test was used. Differences in proportions were analyzed using the chi-square test. The significance of linear associations between two parametric variables was assessed using the method of Pearson. Multivariate, linear regression analysis was also used to determine which clinical parameters were independently associated with histologic findings of CR. All differences were considered significant for $P < 0.05$. The results of parametric data are expressed as mean ± SD. Nonparametric data are expressed as the mean and range. The analysis was carried out using the Statistical Package for the Social Sciences [18].

Results

Patient characteristics

Between the patients who developed CPDAF and controls, there were no statistically significant differences in age, sex, the prevalence of diabetes, the number of major histocompatibility mismatches at the A or B locus, the percent panel reactive antibodies, the incidence of post-transplant acute tubular necrosis, or other clinical characteristics (Table 1). Interestingly, the number of packs of cigarettes smoked daily, multiplied by the number of years smoked (pack-years), was greater at the time of transplant in patients who subsequently had a progressive decline in function. The outcome associated with histological findings in patients with CPDAF was substantially worse compared to the outcome associated with histological findings from controls (Fig. 2). The times that the biopsies were obtained were similar in patients with CPDAF versus controls (Table 2).

Histopathologic features

There were 12 ± 14 glomeruli in each tissue specimen, and there were no differences in the number of glomeruli in specimens from patients who did or did not have CPDAF. Chronic arterial fibrointimal narrowing, arterial intimal proliferation, interstitial fibrosis, glomerular mesangial expansion, tubular atrophy, and a composite CR score were increased in biopsies obtained after the onset of CPDAF (Table 3, Fig. 3). However, these histologic findings were not increased in biopsy specimens obtained before the onset of CPDAF (Table 3). Similar results were observed regarding the proportions of biopsies showing glomerular capillary basement membrane reduplication (Table 3). The number of completely hyalinized glomeruli increased with time after transplantation, but was not different in patients with and without CPDAF (Table 3). Only two biopsies had a significant number of subepithelial deposits; one was in a patient with, and one was in a patient without CPDAF.

Table 1. Clinical characteristics of patients who did or did not have a chronic, progressive decline in allograft function

Characteristic	Progressive decline in function Yes (N = 29)	No progressive decline in function No (N = 45)
Diabetic patients %	31.0	17.8
Second transplant %	10.3	11.1
Males %	55.2	57.8
Native kidneys present %	48.3	55.6
Post-transplant acute tubular necrosis %	44.8	46.7
Highest panel reactive antibodies >50% %	6.9	13.3
Current panel reactive antibodies >50% %	3.4	4.4
Smoked ≥5 pack-years at transplant %	65.5	42.2[a]
Age at transplantation *years*	37 ± 13	40 ± 13
Age of donor kidney *years*	26 ± 12	26 ± 11
Number of AB mismatches	1.4 ± 0.6	1.5 ± 0.7
Number of acute rejections	2.8 ± 1.4	2.1 ± 1.1[a]
Died %	10.3	24.4
Returned to dialysis %	79.3	20.0[b]

Values are means ± SD or percents.
[a] $P < 0.05$, [b] $P < 0.001$

The slope of the regression relationship between the degree of arterial intimal narrowing and the time after transplantation was greater in patients with CPDAF than in control patients (Fig. 4). The intercepts, however, were the same in the two groups (Fig. 4). Thus, arterial fibrointimal narrowing was more severe late in the course of CPDAF, but early after transplantation this histologic parameter was not different in patients who did or did not have CPDAF.

The histopathological findings characteristic of acute cellular rejection, such as, interstitial edema and mononuclear cell infiltrates, were similar in patients with CPDAF and in controls (Table 3). Likewise, a composite acute cellular rejection score was similar in patients with and without CPDAF.

Biopsy findings possibly indicative of acute vascular rejection occurred infrequently and the corresponding mean histologic scores were low (Table 4). Segmental glomerular necrosis was seen in 4 of 44 biopsies of patients with CPDAF versus 3 of 56 biopsies from patients with stable function, vascular necrosis occurred in 1 of 44 biopsies of patients with CPDAF versus 1 of 56 biopsies in patients with stable function, and capillary thrombi occurred in 2 of 44 biopsies of patients with CPDAF versus 3 of 56 biopsies of patients with stable function. Moreover, the differences in glomerular necrosis, vascular necrosis,

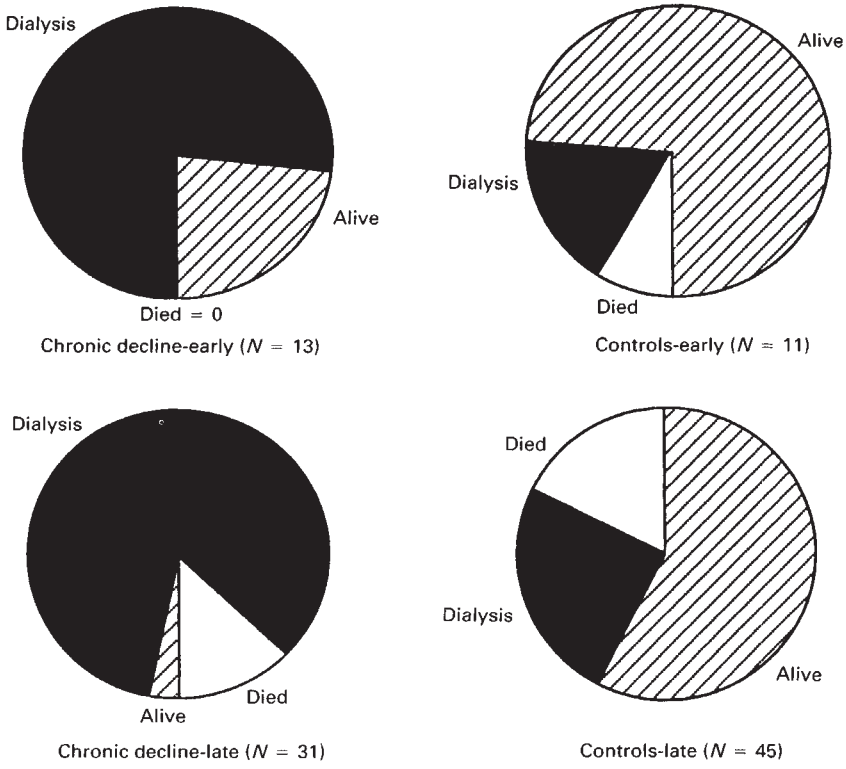

Fig. 2. *Clinical outcome associated with biopsies performed early after transplantation, that is, before the onset of a chronic progressive decline in allograft function (upper left panel), after the onset of a chronic progressive decline in allograft function (lower left panel), early after transplantation in control patients (upper right panel), and late after transplantation in control patients (lower right panel).*

Table 2. Renal function, blood pressure, and proteinuria associated with biopsies obtained from patients with and without a chronic, progressive decline in allograft function

	Progressive decline in function		No progressive decline in function	
	Early (N = 13)	Late (N = 31)	Early (N = 11)	Late (N = 45)
Time of biopsy *months post-transplant*	4 ± 2[a]	45 ± 24[b]	4 ± 3[a]	43 ± 32[b]
C_{Cr} 5 ± 4 months before biopsy *ml/min*	75 ± 19[a]	58 ± 26[b]	71 ± 25[a,b]	75 ± 22[a]
Change in C_{Cr} before biopsy *ml/min*	−37 ± 31[a]	−23 ± 18[a]	−22 ± 29[a]	−25 ± 25[a]
Change in C_{Cr} after biopsy *ml/min*	20 ± 15[a]	−3 ± 12[b]	15 ± 24[a,c]	6 ± 20[b,c]
Mean arterial pressure *mm Hg*	118 ± 11[a]	113 ± 14[a]	111 ± 15[a]	109 ± 16[a]
Hypertension Index	2.2 ± 1.4[a]	2.5 ± 1.8[a]	2.3 ± 1.3[a]	1.9 ± 1.7[a]
Urine protein >1.0 g/24 hr %	2/13 (15%)[a]	10/30 (33%)[a]	1/11 (9%)[a]	8/45 (18%)[a]

Values are mean ± SD except where indicated. Shared superscripts indicate $P > 0.05$.
Abbreviation is: C_{Cr}, creatinine clearance.

and capillary thrombi scores between patients with and without CPDAF were not statistically significant (Table 4). Glomerular endothelial cell swelling was more common in early biopsies, but it was also similar in patients with and without CPDAF (Table 4). Although it was an infrequent finding, interstitial hemorrhage was observed more often early (4 of 13) compared to late (1 of 31) in biopsies from patients with CPDAF, and compared to biopsies from control patients early (1 of 11) and late (1 of 45) after transplantation ($P < 0.01$). Moreover, the interstitial hemorrhage score was greatest in biopsies obtained before the onset of CPDAF (Table 4). The composite acute vascular rejection score was increased in biopsies obtained early after transplantation in patients with and without CPDAF. When the acute vascular rejection scores from early biopsies were analyzed separately, there was a significant difference between patients who subsequently developed CPDAF, 37 (range 0 to 101), and those who did not develop CPDAF 24 (range 0 to 80; $P < 0.01$).

Glomerular area

Glomerular areas increased with time after transplantation. However, there were no differences in glomerular areas from biopsies of patients with CPDAF compared to those from controls (Table 3, Fig. 5). Glomerular areas were not different in biopsies from patients with diabetes (Ln Area = 8.85 ± 0.44) compared to biopsies from patients without diabetes (8.89 ± 0.58).

Correlations between histopathology and renal function

The C_{Cr} at 5 ± 4 months before biopsy was lower in patients with CPDAF compared to controls, but the reduced renal function was evident only in patients who had biopsies in the

Table 3. Histopathologic findings in patients who had a chronic, progressive decline in allograft function or a stable clinical course

Histologic finding	Progressive decline in function		No progressive decline in function		Overall *P* value
	Early (*N* = 13)	Late (*N* = 31)	Early (*N* = 11)	Late (*N* = 45)	
1. Interstitial edema	38 ± 17[a]	38 ± 14[a]	33 ± 18[a]	38 ± 15[a]	0.847
2. Interstitial cells	39 ± 14[a]	46 ± 21[a]	38 ± 19[a]	44 ± 18[a]	0.527
3. Glomerular mesangial expansion	9 ± 11[a]	18 ± 14[b]	10 ± 9[a]	11 ± 7[a]	0.009
4. Chronic arterial intimal occlusion	23 ± 23[a]	47 ± 27[b]	20 ± 18[a]	34 ± 23[a]	0.003
5. Interstitial fibrosis	37 ± 14[a]	63 ± 14[b]	38 ± 16[a]	48 ± 13[c]	<0.001
6. Tubular atrophy	21 ± 17[a]	51 ± 16[b]	21 ± 16[a]	35 ± 16[c]	<0.001
7. Glomerular area (Ln, square microns)	8.5 ± 0.4[a]	9.1 ± 0.7[b]	8.7 ± 0.4[a,b]	9.0 ± 0.5[b]	0.016
8. Arterial intimal proliferation	4 (0–36)	31 (0–85)	1 (0–12)	18 (0–74)	<0.001
9. Glomerulosclerosis %	2 (0–25)	15 (0–75)	1 (0–13)	8 (0–86)	0.008
10. Basement membrane reduplication	1 (0–17)	29 (0–98)	0 (0)	8 (0–84)	0.001
11. Glomerular neutrophil infiltration	8 (0–24)	15 (0–46)	13 (0–25)	11 (0–51)	0.415
12. Acute cellular rejection score (1 + 2)	76 ± 24[a]	83 ± 30[a]	71 ± 33[a]	82 ± 29[a]	0.639
13. Chronic rejection score (3 + 4 + 5 + 6 + 9 + 10)	93 ± 43[a]	223 ± 69[b]	90 ± 54[a]	115 ± 61[c]	<0.001

Values are mean ± SD, or, in the case of non-parametric data, ranges (in parentheses).
[a,b,c] Shared superscripts indicate $P > 0.05$ by analysis of variance and Duncan's multiple range comparison test

late post-transplant period (Table 2). Although the differences were not statistically significant, there was a tendency for the acute decline in C_{Cr} before biopsy to be greater in patients destined to develop CPDAF compared to that seen in controls (Table 2). Biopsy findings typical of CR increased with time after transplantation (Table 5). Histologic findings of CR were also more marked in patients with lower baseline C_{Cr} before biopsy (Table 5). Likewise, C_{Cr} declined immediately after biopsy in patients with CPDAF who underwent biopsy late, but not in patients with CPDAF who underwent biopsy early, and not in controls (Table 2).

Biopsy parameters of acute cellular rejection changed little with time after transplantation (Table 5). Not surprisingly, a greater amount of interstitial edema, or a higher composite acute cellular-rejection score, were associated with a greater acute decline in renal function before biopsy (Table 5). The magnitude of the decline in C_{Cr} before biopsy was also greater in patients who had interstitial hemorrhage (−45 ± 36 ml/min, $N = 7$) compared to those who did not (−25 ± 23 ml/min, $N = 93$, $P < 0.05$). The differences in the decline in C_{Cr} before biopsy between patients who had or did not have glomerular necrosis ($N = 6$), vascular necrosis ($N = 1$), or capillary thrombosis ($N = 4$) were not statistically significant (data not shown).

Diabetes and histopathologic findings

There were 24 biopsies obtained from 17 diabetic patients in the present study. When biopsy findings from diabetics were compared to those from non-diabetics, no differences were found. In particular, the glomerular mesangial expansion score was 10 ± 10 in diabetics versus 14 ± 11 in controls ($P > 0.05$). The fibrointimal arterial occlusion scores were 38 ± 25 and 31 ± 26 in diabetics and nondiabetics, respectively ($P > 0.05$).

Correlations between histopathology, hypertension and proteinuria

The univariate regression analysis showed that few clinical parameters correlated with the histopathologic findings characteristic of acute cellular rejection. A multivariate regression analysis was also carried out using the composite acute cellular rejection score as the dependent variable, and multiple clinical parameters as independent variables. None of the variables examined had a statistically significant impact on the acute cellular rejection score including: the time of biopsy after transplant, the number of acute rejection episodes between the time of transplant and the time of biopsy, the number of A or B major histocompatibility antigen mismatches, the presence of preformed antibodies, and acute tubular necrosis post-transplant.

A number of clinical parameters were associated with histologic findings of CR. Most closely correlated with the composite CR score was the time of biopsy (Table 5). However, there was also a strong correlation between the CR score and the number of acute rejections before the time of biopsy ($r = 0.47$, $P < 0.001$). The Hypertension Index also correlated with the CR score ($r = 0.38$, $P < 0.001$ before biopsy, $r = 0.33$, $P < 0.001$ at the time of biopsy, and $r = 0.26$, $P < 0.05$ after biopsy). The proportion of patients with urine protein excretion greater than 1.0 g/24 hr was almost twofold greater in patients with CPDAF compared to controls, but this difference was not statistically significant (Table 2). Nevertheless, patients with urine protein excretion greater than 1.0 g/24 hr had more intrarenal vascular damage, interstitial fibrosis, glomerular basement membrane reduplication and subepithelial deposits compared to patients who did not have proteinuria (Table 6).

Multivariate analysis was carried out using the CR score as the dependent variable, and multiple clinical parameters as independent variables. Post-transplant variables known to be associated with chronic renal damage, such as proteinuria and hypertension, were excluded from this analysis to avoid parameters that could have been the result rather than the cause of CR. Interestingly, the time of biopsy, the number of acute rejection episodes before biopsy and cigarette smoking were each independently correlated with the CR score = 0.20(MO) + 4.00(AR) + 0.13(PY) + 16.23, where MO was the time of biopsy in months post-transplant, AR was the number of acute rejection episodes before biopsy, and PY was the number of pack-years of cigarettes smoked at the time of transplant. These variables explained 41% of the variability in the CR score ($P < 0.001$). Variables that had no impact on the CR score included

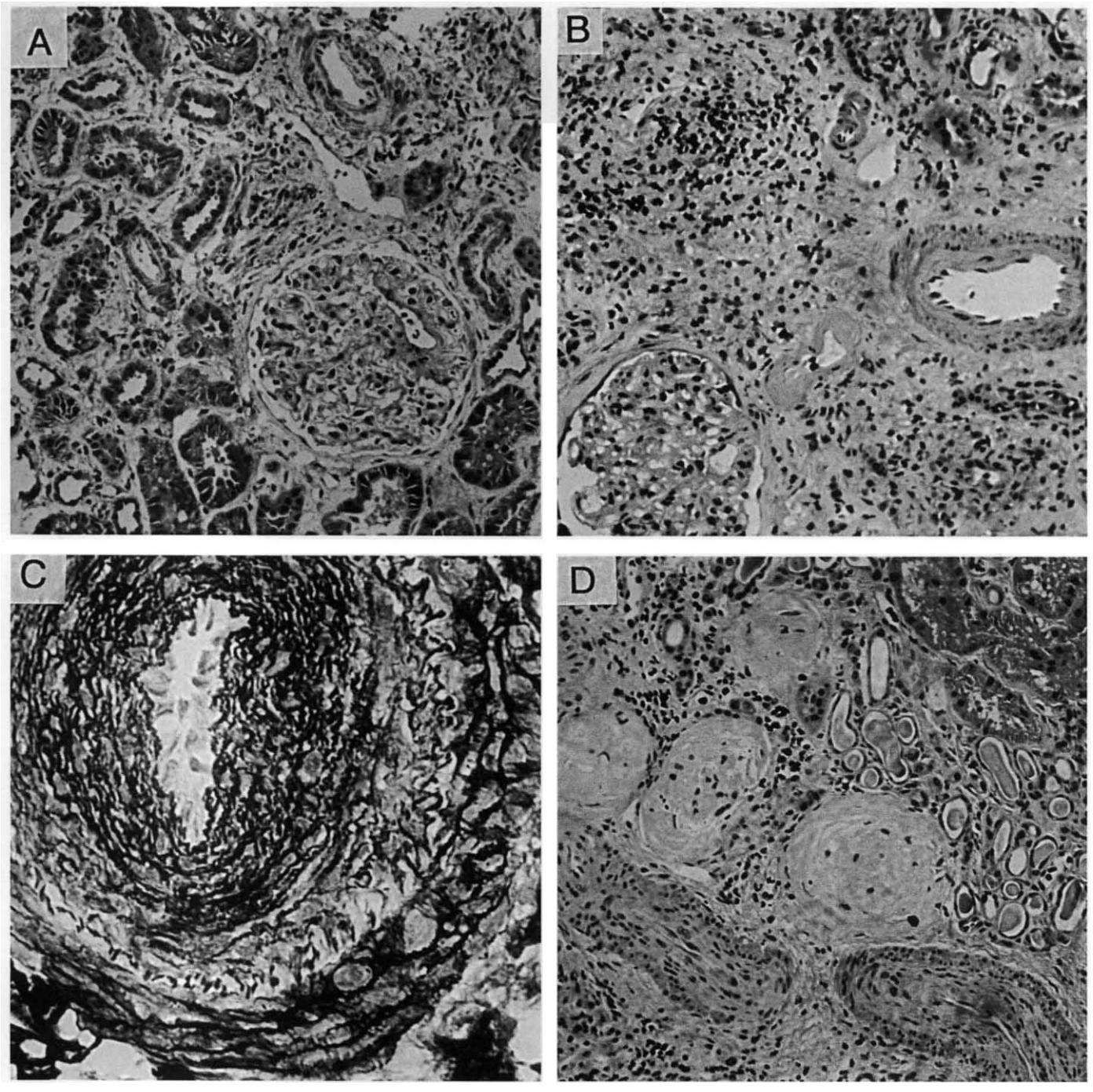

Fig. 3. *Biopsy findings associated with a chronic, progressive decline in allograft function.* **A** Mild-moderate interstitial fibrosis (Masson's trichrome). **B** Marked interstitial fibrosis, acute interstitial infiltrate, and mild fibrointimal arterial disease (hematoxylin-eosin). **C** Severe, fibrointimal, occlusive arterial disease (Jones' modification of the periodic acid-methenamine silver stain). **D** Severe fibrointimal, vascular occlusive disease with several hyalinized glomeruli, and a marked degree of interstitial fibrosis (hematoxylin-eosin). Original magnification: 400× in panel A, 200× in panels B, C, and D.

the number of A or B major histocompatibility antigen mismatches, the presence of preformed antibodies, age, donor age, sex, donor sex, diabetes, the occurrence of acute tubular necrosis after transplantation or the presence of systemic cardiovascular disease at the time of transplant.

Histopathologic correlates of subsequent graft survival time

The 46 patients who returned to dialysis during the study period were analyzed separately to examine how well allograft histology correlated with the duration of subsequent graft

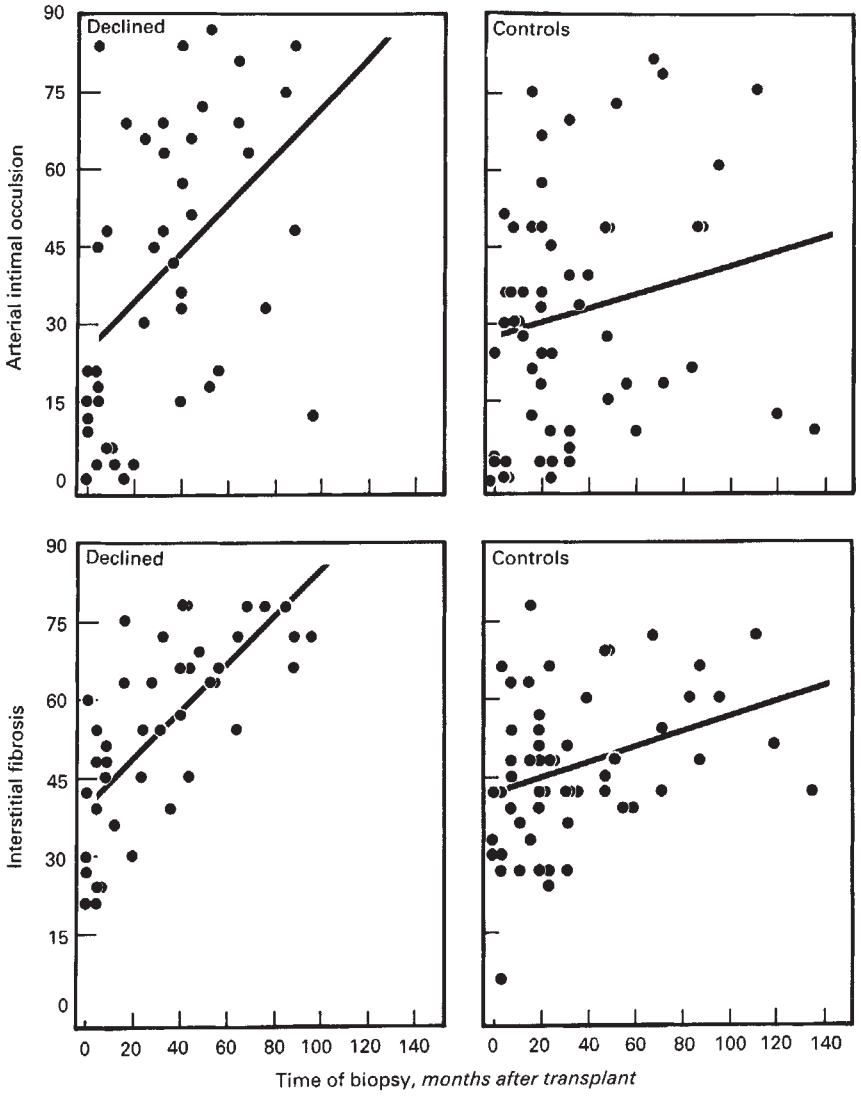

Fig. 4. *The degree of fibrointimal arterial occlusion in patients who had a chronic progressive decline in allograft function (CPDAF) (upper left panel; score = 0.47 (T) + 24, where T = months after transplantation, P < 0.01) and in control patients (upper right panel; score = 0.15 (T) + 26, P = 0.11).* The slopes were significantly different indicating a more rapid increase in the degree of vascular occlusion after transplantation in patients with CPDAF. The intercepts were not different, indicating that the degree of chronic vascular disease in biopsies obtained early after transplantation failed to predict patients who developed CPDAF. The degree of interstitial fibrosis in patients who had CPDAF (lower left panel; score = 0.44 (T) + 41, $P < 0.001$) and in control patients (lower right panel; score = 0.15 (T) + 41, $P < 0.05$).

Table 4. Histopathologic findings possibly indicative of acute vascular rejection in patients who had a chronic progressive decline in allograft function or a stable clinical course

Histologic finding	Progressive decline in function		No progressive decline in function		Overall *P* value
	Early (*N* = 13)	Late (*N* = 31)	Early (*N* = 11)	Late (*N* = 45)	
1. Interstitial hemorrhage	4 (0–18)	0 (0–2)	0 (0–1)	1 (0–23)	0.003
2. Glomerular endothelial swelling	25 (0–87)	11 (0–58)	22 (0–68)	6 (0–72)	0.027
3. Glomerular necrosis	1 (0–7)	1 (0–19)	2 (0–12)	2 (0–77)	0.277
4. Vascular necrosis	5 (0–60)	0 (0)	0 (0)	1 (0–41)	0.385
5. Capillary thrombosis	3 (0–24)	0 (0)	0 (0)	2 (0–80)	0.143
6. Acute vascular rejection score (1 + 2 + 3 + 4 + 5)	37 (0–101)	12 (0–58)	24 (0–80)	11 (0–200)	0.004

Values are means and (in parentheses) ranges.

survival. Findings characteristic of acute cellular rejection were not associated with the number of months remaining before patients resumed dialysis (Table 6). The time remaining until return to dialysis was also similar in patients with and without biopsy findings of acute vascular rejection. For example, among patients who returned to dialysis, the survival time after biopsies showing interstitial hemorrhage ($N = 5$) was 41 ± 27 months compared to 31 ± 27 months ($P > 0.05$) if biopsies did not have interstitial hemorrhage ($N = 41$). The survival after biopsies showing any feature of acute vascular rejection ($N =$

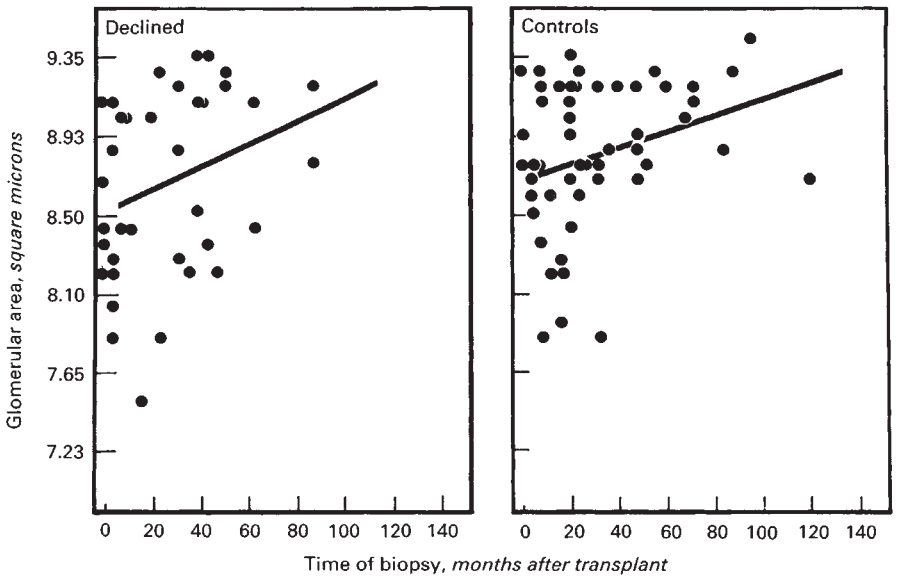

Fig. 5. *The area inside Bowman's capsule (logarithmically transformed) in patients with a chronic progressive decline in allograft function (CPDAF) (area is the logarithm of square microns = 0.011 (T) + 8.5, where T = months after transplantation, P < 0.001) and in patients who maintained stable function (area = 0.006 (T) + 8.7, P < 0.001).* The slopes were not different ($P > 0.05$), indicating that the increases in glomerular areas during the post-transplant period were not significantly different in patients with and without CPDAF.

Table 5. Relationship (*r* values) between the time of biopsy, renal function, and histopathologic findings

Histopathologic features	Time of biopsy *months after transplant*	C_{Cr} 5 ± 4 months before biopsy *ml/min*	Change in C_{Cr} before biopsy %	Change in C_{Cr} after biopsy %
Interstitial edema	−0.12	0.27[b]	−0.40[c]	0.14
Interstitial cells	−0.04	0.11	−0.10	−0.10
Glomerular mesangial expansion	0.17	−0.31[b]	0.02	0.04
Chronic arterial intimal thickening	0.31[b]	−0.27[b]	0.13	−0.20[a]
Interstitial fibrosis	0.45[c]	−0.35[c]	0.14	−0.27[b]
Tubular atrophy	0.35[c]	−0.16	0.01	−0.28[b]
Glomerular area[d]	0.45[c]	−0.25[a]	0.10	−0.06
Acute cellular rejection score[e]	−0.09	0.22[a]	−0.27[b]	0.01
Chronic rejection score[e]	0.56[c]	−0.40[c]	0.09	−0.30[b]

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$
[d] Glomerular areas were logarithmically (Ln) transformed
[e] Acute cellular and chronic rejection scores are defined in Table 3

23) was 30 ± 26 months compared to 34 ± 29 months ($P > 0.05$) if biopsies had no features of acute vascular rejection ($N = 23$). In contrast, the histopathologic features of CR were highly correlated with the subsequent graft survival time. The best predictor of the duration of subsequent graft survival was the composite CR score (Table 6).

Discussion

Over the past three decades great strides have been made in the prevention and treatment of acute renal allograft rejection. The success of this effort has led to a substantial decrease in early allograft failure and patient mortality. However, with the reduction in the incidence of acute rejection episodes and early graft failure, CR and late graft loss have become increasingly problematic [19].

While several investigations have correlated biopsy findings with the course and outcome after acute rejection [1–6], few studies have examined the relationship between the renal histology of CR and the clinical course of declining allograft function. In some investigations, fibrointimal arterial lesions seen within the first three months after transplantation were associated with rapidly progressive allograft failure [7]. On the other hand, similar arterial lesions have been found in allograft biopsies obtained in the late post-transplant period [8–10]. The frequency with which fibrointimal arterial occlusion and other histologic findings of CR are associated with CPDAF has not been well defined.

In the present investigation histologic findings of CR were seen after, but not before, the onset of CPDAF. The histopathologic findings that were most closely associated with CPDAF were interstitial fibrosis, glomerular mesangial expansion, chronic, fibrointimal arterial occlusion, and tubular atrophy/dropout. Other findings were more frequent in patients with CPDAF, but occurred too infrequently to be reliable indicators of CPDAF. Glomerular basement membrane reduplication, for example, was seen in 48% of the biopsies in patients with CPDAF versus 18% of the biopsies in patients who maintained stable allograft function ($P < 0.05$). The best overall indicator of CPDAF was the composite CR score (fibrointimal arterial narrowing + interstitial fibrosis + tubular atrophy + the percent of glomeruli that were hyalinized + glomerular basement membrane reduplication).

Table 6. Clinical and histopathologic findings associated with proteinuria

Histopathologic features	Urine protein excretion >1.0 g/24 hr at the time of biopsy	
	No (N = 78)	Yes (N = 21)
Interstitial edema	38 ± 15	32 ± 13
Interstitial cells	44 ± 20	39 ± 12
Glomerular mesangial expansion	11 ± 9	17 ± 16
Chronic arterial intimal thickening	32 ± 25	46 ± 23[a]
Interstitial fibrosis	47 ± 17	61 ± 13[b]
Tubular atrophy	35 ± 20	41 ± 17
Basement membrane reduplication	7 (0–94)	31 (0–98)[b]
Subepithelial deposits	0.0 (0)	0.1 (0–1.0)[b]
Acute cellular rejection score[c]	82 ± 31	71 ± 18
Acute vascular rejection score[d]	14 (0–101)	23 (0–200)[b]
Chronic rejection score[c]	140 ± 71	209 ± 76[b]

Values are mean ± SD except where indicated, or in the case of non-parametric data, ranges (in parentheses).

[a] $P < 0.05$; [b] $P < 0.01$

[c] Acute cellular rejection and chronic rejection scores are defined in Table 3

[d] Acute vascular rejection score is defined in Table 4

Table 7. Relationship (r values) between histopathologic features and the subsequent allograft survival time in 46 patients who returned to dialysis

Histopathologic features	Time remaining before return to dialysis *months*
Interstitial edema	0.24
Interstitial cells	−0.04
Glomerular mesangial expansion	−0.31[a]
Chronic arterial intimal thickening	−0.44[b]
Interstitial fibrosis	−0.54[c]
Tubular atrophy	−0.44[b]
Acute cellular rejection score[d]	0.05
Chronic rejection score[d]	−0.65[c]

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$

[d] Acute cellular rejection and chronic rejection scores are defined in Table 3

The results of the present investigation are in general agreement with those of another recent study that examined the relationship between histopathologic findings and combined patient and graft survival [5]. In that study, features such as fibrointimal thickening of arteries and arterioles were associated with a poor outcome. Interestingly, the increased incidence of combined patient and graft loss associated with chronic vascular lesions in that study was limited to the three month period after the biopsy was obtained [5]. In the present study, patients who underwent biopsy after the onset of CPDAF survived with functioning allografts for 1.9 ± 1.9 years (95% confidence intervals = 1.2 to 2.6 years) after biopsy. Thus, in the present investigation, the histologic lesions of CR were seen in patients whose grafts continued to function for an extended period of time. Moreover, there was a good correlation between the severity of the CR biopsy findings and the duration of subsequent allograft survival.

The pathogenesis of CR is unknown. Although immunologic factors may be important, there is little evidence that CR is entirely mediated by immunologic events [14, 20]. Recent experimental data have suggested that a reduction in the amount of functioning renal mass leads to compensatory changes in the remaining intact nephrons that may ultimately be injurious. Increases in single nephron glomerular filtration rate and plasma flow, compensatory growth and hyperplasia of intact nephrons, and other factors resulting from a reduced nephron mass have been suggested to lead to progressive renal injury [21–25].

In the present study, the size of intact glomeruli increased with time after transplantation. Interestingly, the increase in glomerular area did not seem to be confined to the immediate post-transplant period (Fig. 5). Even more interesting was the fact that the increase in the glomerular size was similar in biopsies from patients with CPDAF compared to controls. Thus, it is difficult to implicate factors associated with compensatory growth and hyperplasia of residual glomeruli in the pathogenesis of CPDAF. Why there was not a greater increase in the size of intact glomeruli in patients with CPDAF is unclear. However, it is possible that the fibrointimal vascular occlusion associated with CR may have limited the normal, compensatory, hypertrophic response.

Since intrarenal vascular disease is one of the most striking features of CR, the relationship between known systemic vascular disease risk factors and CR was carefully examined in the present study. Hypertension was common and more severe in patients with CR. To what extent the hypertension was a cause and/or a result of the renal damage is unclear. However, the fibrointimal occlusion associated with CR tended to be most marked in large intrarenal arteries. In native kidneys, on the other hand, hypertension characteristically affects smaller arteries and arterioles [26, 27]. Thus, although hypertension may have contributed to the fibrointimal vascular damage seen in patients with CR, the prominent involvement of large intrarenal arteries suggests that these lesions were not due to hypertension alone.

Proteinuria was also common in the present study, and patients with proteinuria were more likely to have histologic findings of CR. Whether proteinuria directly, or indirectly, contributed to the development and progression of CR is unknown. It is interesting to speculate, however, that increased serum lipids associated with proteinuria could have contributed to both glomerular and vascular damage in CR.

Regression analysis using clinical parameters measured at the time of biopsy cannot distinguish potential causes from possible consequences of CR. Thus, we can only speculate whether clinical variables such as hypertension, hyperlipidemia and proteinuria were important in the pathogenesis of CR. However, we examined the possible impact of other variables measured before biopsy. Only the time of biopsy, the number of acute rejection episodes before biopsy, and cigarette smoking at the time of transplantation were independently correlated with the histologic findings of CR. The association between cigarette smoking and CR has not been previously reported, and provides further support to the hypothesis that risk factors for systemic vascular disease may also be risk factors for CR.

In a previous investigation of renal function after transplantation, we found no clinical or laboratory parameters that were reliable predictors of patients destined to develop CPDAF [16]. In the present study, the histologic findings of acute cellular rejection and CR did not precede the onset of CPDAF. However, biopsies obtained before the onset of CPDAF were more likely to have interstitial hemorrhage, a sign of severe, acute, vascular rejection. These results are consistent with those of another investigation that found patients who had severe acute rejection episodes in the early post-transplant period were more likely to develop chronic rejection [28]. Although it may cause CR, acute vascular rejection occurs infrequently, and, thus, heralds the onset of CPDAF in only a small number of patients.

In the present investigation, the best overall histologic correlate to the degree of acute functional decline before biopsy was the amount of interstitial edema. There was no correlation between the amount of interstitial cellular infiltrate and the magnitude of decline in renal function before biopsy. Similarly, there was no difference in the degree of acute, interstitial, cell infiltrate in patients who did or did not have CPDAF. These results are in agreement with those of others who found that the degree of acute, interstitial, cell infiltrate did not correlate with the clinical course [1, 29].

The patients in the present study were treated with the same conventional immunosuppression protocol. As a result it was not possible to investigate the relative impact of the individual immunosuppressive agents on allograft histology. Moreover, since none of the patients in the present study received cyclosporin A (CsA), the possible impact of CsA on the histopathology of CR cannot be determined from these results. On the other hand, the fact that none of the patients were treated with CsA permits us to conclude that the histologic damage and CPDAF were caused by immune and/or nonimmune mechanisms unrelated to chronic CsA nephrotoxicity. In the future, it may be difficult to clearly distinguish chronic intrarenal vascular damage and interstitial fibrosis caused by CsA from that caused by CR.

In summary, the biopsy findings of CR were seen after, but not before, the onset of CPDAF, suggesting that the histopathology features of CR may not be useful in predicting CPDAF. On the other hand, the results confirm that the histologic features of CR were more common in patients with CPDAF, and indicate that a histologic diagnosis of CR may be associated with a very gradual deterioration in renal function. Thus, in patients with CPDAF, a biopsy may be useful in confirming the diagnosis of CR, and in excluding other causes of allograft dysfunction. Moreover, the severity of the chronic histopathologic changes may predict the duration of subsequent allograft survival. Finally, the associations between hypertension, cigarette smoking and CR suggest vascular disease risk factors could be important in the pathogenesis of CR.

Acknowledgments

Dr. Kalil is a recipient of a scholarship (201035/89-8) from Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), Ministerio da Ciencia e Tecnologia, Brazil. Our sincere thanks goes to Ms. Jane Gharbi for her invaluable assistance in collecting the data for this study, and to Ms. Jan Lovick and Ms. Dee Lunzer for helping to prepare the manuscript.

Reprint requests to Bertram L. Kasiske, M.D., Department of Medicine, Hennepin County Medical Center, 701 Park Avenue, Minneapolis, Minnesota 55439, USA.

References

1. Waltzer WC, Miller F, Arnold A, Anaise D, Rapaport FT: Immunohistologic analysis of human renal allograft dysfunction. *Transplantation* 43:100–105, 1987
2. Banfi G, Imbasciati E, Tarantino A, Ponticelli C: Prognostic value of renal biopsy in acute rejection of kidney transplantation. *Nephron* 28:222–226, 1981
3. Parfrey PS, Kuo YL, Hanley JA, Knaack J, Xue Z, Lisbona R, Guttmann RD: The diagnostic and prognostic value of renal allograft biopsy. *Transplantation* 38:586–590, 1984
4. Herbertson BM, Evans DB, Calne RY, Banerjee AK: Percutaneous needle biopsies of renal allografts: the relationship between morphological changes present in biopsies and subsequent allograft function. *Histopathology* 1:161–178, 1977
5. Matas AJ, Sibley R, Mauer M, Sutherland DER, Simmons RL, Najarian JS: The value of needle renal allograft biopsy. I. A retrospective study of biopsies performed during putative rejection episodes. *Ann Surg* 197:226–237, 1983
6. Finkelstein FO, Siegel NJ, Bastl C, Forrest JN Jr, Kashgarian M: Kidney transplant biopsies in the diagnosis and management of acute rejection reactions. *Kidney Int* 10:171–178, 1976
7. Porter KA, Thomson WB, Owen K, Kenyon JR, Mowbray JF, Peart WS: Obliterative vascular changes in four human kidney homotransplants. *Brit Med J* 2:639–645, 1963
8. Porter KA, Rendall JM, Stolinski C, Terasaki PI, Marchioro TL, Starzl TE: Light and electron microscopic study of biopsies from thirty-three human renal allografts and an isograft 1¾–2½ years after transplantation. *Ann NY Acad Sci* 129:615–636, 1966
9. Busch GJ, Galvanek EG, Reynolds ES Jr: Human renal allografts: analysis of lesions in long-term survivors. *Hum Pathol* 2:253–298, 1971
10. Petersen VP, Olsen TS, Kissmeyer-Neilsen F, Bohman S-O, Hansen HE, Hansen ES, Skov PE, Sølling K: Late failure of human renal transplants. An analysis of transplant disease and graft failure among 125 recipients surviving for one to eight years. *Medicine* 54:45–71, 1975
11. Olsen S, Bohman S-O, Petersen VP: Ultrastructure of the glomerular basement membrane in long term renal allografts with transplant glomerular disease. *Lab Invest* 30:176–189, 1974
12. Rao KV, Rose JK: Incidence, histological pattern, and clinical outcome of rejection episodes occurring in the late post-transplant period. *Transplantation* 40:631–637, 1985
13. Abbud-Filho M, Ramalho HJ, Barberato JB, Corrente JE, Zerati-Filho M, Verona CBM, Martucci RC, Bezas AG: Inhibition of chronic kidney allograft rejection by cyclosporine. *Transplant Proc* 21:1660–1662, 1989
14. Feehally J, Harris KP, Bennett SE, Walls J: Is chronic renal transplant rejection a non-immunological phenomenon? *Lancet* 2:486–488, 1986
15. Kootte AMM, 'T Hart-Eerdmans M, Paul LC: Dietary protein manipulation or cyclosporin therapy in chronic renal allograft rejection. *Clin Transplant* 2:152–159, 1988
16. Kasiske BL, Heim-Duthoy KL, Tortorice KL, Rao KV: The variable nature of chronic declines in renal allograft function. *Transplantation* 51:330–334, 1991
17. Jones RH, Molitoris BA: A statistical method for determining the breakpoint of two lines. *Anal Biochem* 141:287–290, 1984
18. Norusis MJ: *Statistical Package for the Social Sciences (SPSS/PC+ V2.0): Base Manual.* Chicago, SPSS Inc., 1988
19. Foster MC, Wenham PW, Rowe PA, Burden RP, Morgan AG, Cotton RE, Blamey RW: The late results of renal transplantation and the importance of chronic rejection as a cause of graft loss. *Ann R Coll Surg Engl* 71:44–47, 1989
20. Fellström B, Larsson E, Tufveson G: Strategies in chronic rejection of transplanted organs: A current view on pathogenesis, diagnosis, and treatment. *Transplant Proc* 21:1435–1439, 1989

21. HOSTETTER TH, OLSON JL, RENNKE HG, VENKATACHALAM MA, BRENNER BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
22. FRIES JW, SANDSTROM DJ, MEYER TW, RENNKE HG: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest* 60:205–218, 1989
23. HARRIS DCH, CHAN L, SCHRIER RW: Remnant kidney hypermetabolism and progression of chronic renal failure. *Am J Physiol* 254:F267–F276, 1988
24. KASISKE BL, O'DONNELL MP, GARVIS WJ, KEANE WF: Pharmacologic treatment of hyperlipidemia reduces glomerular injury in rat 5/6 nephrectomy model of chronic renal failure. *Circ Res* 62:367–374, 1988
25. FOGO A, HAWKINS EP, BERRY PL, GLICK AD, CHIANG ML, MACDONELL RC JR, ICHIKAWA I: Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int* 38:115–123, 1990
26. WILLIAMS RH, HARRISON TR: A study of the renal arteries in relation to age and to hypertension. *Am Heart J* 14:645–658, 1937
27. YAMAGUCKI T, OMAE T, KATSUKI S: Quantitative determination of renal vascular changes related to age and hypertension. *Jpn Heart J* 10:248–258, 1969
28. FOSTER MC, ROWE PA, DENNIS MJS, MORGAN AG, BURDEN RP, BLAMEY RW: Characteristics of cadaveric renal allograft recipients developing chronic rejection. *Ann R Coll Surg Engl* 72:23–26, 1990
29. BURDICK JF, BESCHORNER WE, SMITH WJ, MCGRAW D, BENDER WL, WILLIAMS GM, SOLEZ K: Characteristics of early routine renal allograft biopsies. *Transplantation* 38:679–684, 1984